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State-wide increase in prenatal diagnosis of klinefelter syndrome on amniocentesis and chorionic villus sampling: Impact of non-invasive prenatal testing for sex chromosome conditions

Lulu Loughry[1,2] | Cecilia Pynaker[1] | Mary White[1,2,3,4] | Jane Halliday[1,2] | Lisa Hui[1,5,6,7]

[1]Reproductive Epidemiology Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[2]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[3]Department of Endocrinology & Diabetes, The Royal Children's Hospital, Parkville, Victoria, Australia

[4]Health Services Research Unit, The Royal Children's Hospital, Parkville, Victoria, Australia

[5]Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia

[6]Department of Perinatal Medicine, Mercy Hospital for Women, Heidelberg, Victoria, Australia

[7]Department of Obstetrics and Gynaecology, Northern Health, Epping, Melbourne, Victoria, Australia

Correspondence
Lisa Hui, Reproductive Epidemiology Group, Murdoch Children's Research Institute, Flemington Rd, Parkville, Victoria 3052, Australia.
Email: lisa.hui@mcri.edu.au

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Abstract

Background: To analyze population-based trends in the prenatal diagnosis of sex chromosome aneuploidy (SCA) since the availability of non-invasive prenatal testing (NIPT).

Methods: Retrospective state-wide data for all prenatal diagnoses performed <25 weeks gestation from 2005 to 2020 in Victoria, Australia. Non-invasive prenatal testing became locally available from 2012. The prenatal diagnosis rates of SCA as proportions of all prenatal diagnostic tests and all births were calculated. Statistical significance was assessed with the χ^2 test for trend, with $p < 0.05$ considered significant.

Results: 46,518 amniocentesis and chorionic villus sampling were performed during the study period, detecting 617 SCAs. There was a significant increase in the rate of prenatal SCAs from 5.8 per 10,000 births in 2005 to 8.7 per 10,000 births in 2020 ($p < 0.0001$). This increase was predominantly due to 47,XXY cases, 91% of which were ascertained via positive NIPT for this condition in 2020. The prenatal diagnosis rate of 47,XXY significantly increased from 0.8 per 10,000 births in 2005 to 4.3 per 10,000 births in 2020 ($p < 0.0001$).

Conclusion: Screening for SCAs using NIPT has directly led to an increase in their prenatal diagnosis on a population-wide basis, especially 47,XXY. This has implications for clinician education, genetic counselling, and pediatric services.

Key points

What is already known about this topic?

- Noninvasive prenatal testing for fetal aneuploidy has created new opportunities for the identification of sex chromosome conditions without an ultrasound phenotype.

These results were presented at the Virtual Annual Scientific Meeting of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists in February 2021

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What does this study add?

- This is the first population-based data demonstrating the significant rise in prenatal diagnosis of sex chromosome conditions on amniocentesis and chorionic villus sampling, predominantly driven by increases in the prenatal diagnosis of 47,XXY.
- The prenatal diagnosis rate of 47,XXY has increased from one in 12,500 births to one in 2300.
- This five-fold increase in the prenatal diagnosis of 47,XXY has significant clinical implications for health professional education and postnatal management.

1 | INTRODUCTION

Until recently, the prenatal diagnosis of a sex chromosome condition was most commonly an incidental finding following a diagnostic procedure performed for another indication.[1] The introduction of NIPT over the past decade has not only reduced the total number of invasive procedures performed,[2] but also created new opportunities for women to obtain specific prenatal screening information on SCAs. Non-invasive prenatal testing became clinically available in Australia on a self-funded basis in late 2012[3] and is now utilized by at least 20% of women electing to have screening in Victoria.[4]

The most common SCA, 45,X (Turner syndrome), is the only sex chromosome aneuploidy (SCA) with the potential for detection via specific fetal ultrasound findings (cystic hygroma, hydrops fetalis, increased nuchal translucency, or cardiac and renal anomalies). The other SCAs include 47,XXY (Klinefelter syndrome), 47,XYY (Jacob Syndrome) and 47,XXX (Triple X). Children with these conditions often have a normal phenotype at birth typically presenting across the life course with issues including developmental delay, reproductive abnormalities, and reduced fertility.[5] Their highly variable presentation and the less accurate performance of NIPT for these conditions has created debate over whether sex chromosomes should be analyzed in NIPT.[6] A joint statement by The Royal Australian and New Zealand College for Obstetricians and Gynecologists and the Human Genetics Society of Australasia recommended that while there is no precedent for screening for SCA at the population level, if it is to be offered it must be done in a voluntary manner, with informed consent, including counselling about possible unanticipated findings and the option to opt out.[7] It is notable that in some countries, such as India and China, assessment of fetal sex chromosomes is not legally permitted due to concerns about sex selection.[8] Furthermore, there is significant variation in the range of conditions for which screening by NIPT is done, not only between countries, but also between different providers within the same state or country.[9] This ranges from only screening for chromosomes 13, 18, and 21, to genome wide analysis including rare autosomal trisomies +/− sex chromosomes, as seen in countries such as Belgium and the Netherlands.[9,10]

We used a population-based prenatal diagnosis data collection to analyze trends in the definitive prenatal diagnosis of SCA on amniocentesis and CVS before and after the introduction of NIPT in 2012. We hypothesized that the prenatal diagnosis of SCAs, and 47,XXY specifically, would rise significantly after the clinical availability of NIPT.

2 | MATERIALS AND METHODS

The Australian state of Victoria has approximately 79,000 births annually, with an average fertility rate of 1.7 births per woman and a median maternal age of 31.5 years[11] Women in the most advantaged regions of Victoria have a higher rate of prenatal diagnosis, with 315 prenatal diagnoses performed per 10,000 births compared to 149 per 10,000 births for women in the most disadvantaged regions.[12] Prior work from our group has shown that advantaged regions are also five times more likely to have NIPT-indicated prenatal diagnosis compared with women from disadvantaged regions.[13]

The Victorian Prenatal Diagnosis Database (VPDD) is a centralized data collection for prenatal diagnosis that captures all such testing in our population. All CVS and amniocentesis samples collected at <25 weeks gestation from 2005 to 2020 were included in this study. Indications for testing were provided by the clinical referrer. Chromosomal analysis was performed by G-banded karyotyping, fluorescence in situ hybridization, and/or chromosomal microarray. Multiple pregnancies and repeat tests were merged into single 'per pregnancy' records. Data were analyzed using STATA version 16 (StataCorp, 2019) and EpiTools (2018).[14]

Annual numbers of SCAs as a percentage of total prenatal diagnostic tests were analyzed with a χ^2 test for trend with a p value of <0.05 being considered significant. Annual numbers of Victorian registered births were obtained from government sources to calculate rates of prenatal diagnoses of SCAs per 10,000 births during 2005–2020.[11,15] The prenatal diagnosis results and the birth registrations were not linked, so the rate per 10,000 births is not able to capture true prevalence. However, this metric was used to control for variation in annual birth rates.

This study received Human Research Ethics Committee (HREC) approval from the Royal Children's Hospital HREC (Reference No. 31135A) and Monash Health HREC (Reference No. 12063B). A waiver of individual patient consent was granted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research 2007 Section 2.3.10.

3 | RESULTS

During the 16-year study period there were 1,117,475 births and 46,518 prenatal diagnostic procedures, identifying 617 SCAs. The most common SCA detected during the study period was 45,X ($n = 294$), followed by 47,XXY ($n = 174$), 47,XXX ($n = 91$) and 47,XYY ($n = 53$). The annual number of SCAs remained relatively stable between 2005 and 2015, ranging between 20 and 41 per annum, after which an upward trend in annual cases to a peak of 65 in 2020 was observed (Figure 1A, Supplementary Material S1). The total number of confirmed SCAs as a percentage of prenatal diagnostic tests increased significantly over the total study period from 0.8% in 2005% to 3.3% in 2020 (χ^2 trend = 279.6, $p < 0.0001$). When analyzed as proportion of total births, the prenatal diagnosis rate of an SCA increased from 5.8 per 10,000 births in 2005 to 8.7 per 10,000 births in 2020 (χ^2 trend = 45.9, $p < 0.0001$) (Figure 1B).

This rise in SCAs was largely driven by a significant increase in the identification of 47,XXY cases. Since 2017, 47,XXY has exceeded 45,X as the most common SCA detected on prenatal diagnosis (Supplementary Material S1). The prenatal diagnosis rate of 47,XXY significantly increased from 0.8 per 10,000 births in 2005 to 4.3 per 10,000 births in 2020 (χ^2 trend = 45.2, $p < 0.0001$). This translates to a prenatal diagnosis rate of 8.4 per 10,000 male births in 2020.

Non-invasive prenatal testing is the now most common screening method leading to a prenatal diagnosis of SCA (Figure 2). More than 80% of all SCA were ascertained via NIPT in 2020, including 29 of 32 cases of 47,XXY (91%) (Table 1).

4 | DISCUSSION

Using a population-based data collection with full capture of amniocentesis and CVS procedures, we show an unprecedented rise in the prenatal diagnosis of SCA since 2016, predominantly driven by detection of 47,XXY via NIPT. While NIPT became available on a self-funded basis in late 2012, there was no statistically significant

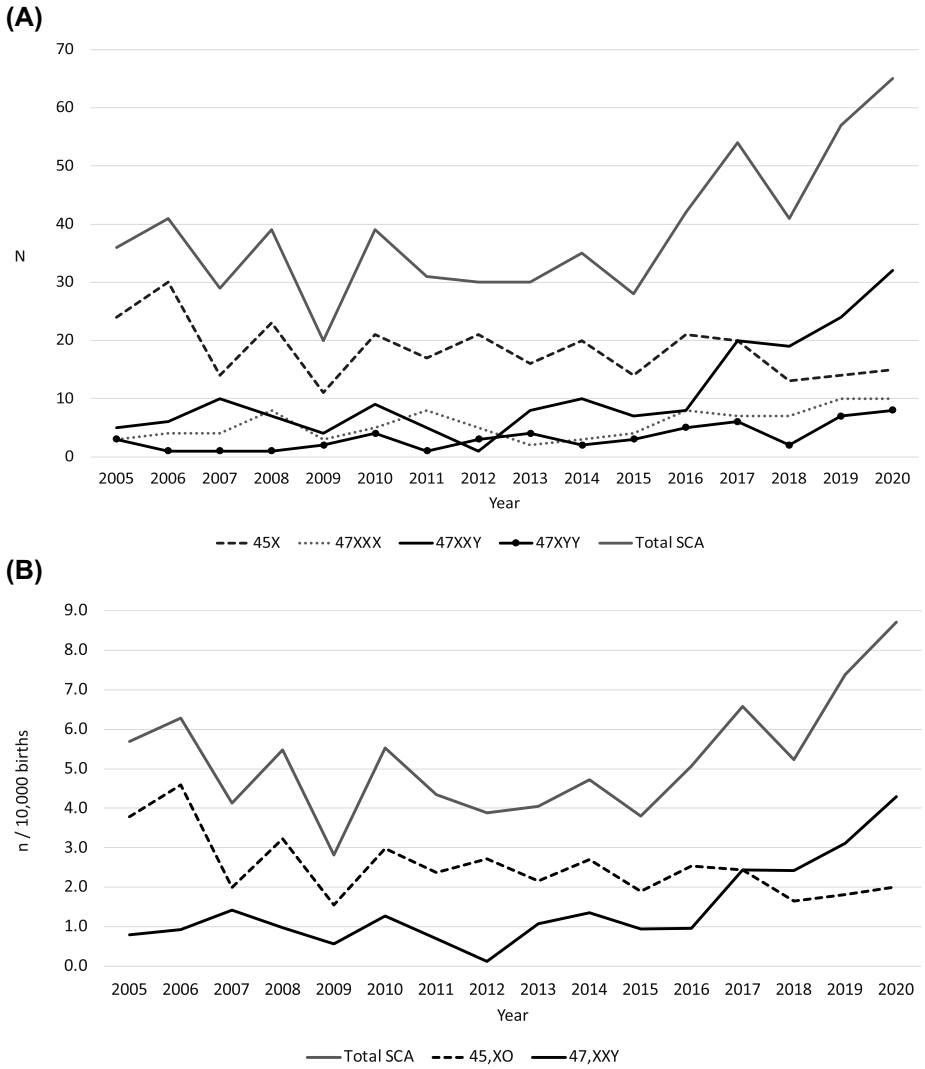

FIGURE 1 Annual prenatal diagnoses of sex chromosome aneuploidy (SCA) 2005–2020 (A). Frequency of SCA (B). Rate of SCA per 10,000 births in Victoria. SCA, sex chromosome aneuploidy

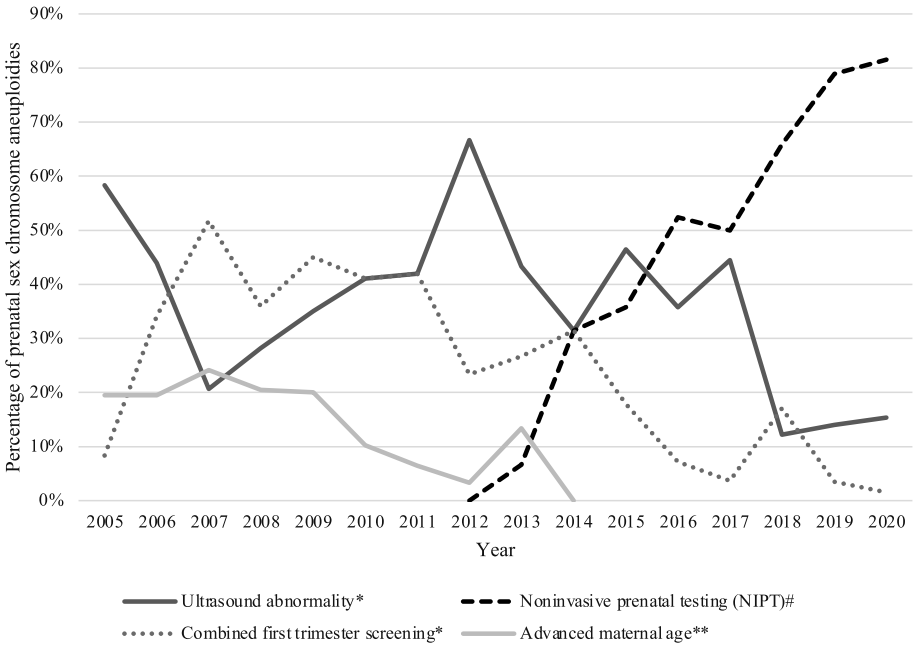

FIGURE 2 Indications for prenatal diagnosis in cases of confirmed sex chromosome aneuploidy (SCA) (2005–2020). #20 cases also had an ultrasound abnormality (12 structural, 8 increased nuchal translucency). *22 cases also had an ultrasound abnormality (21 structural, 1 increased nuchal translucency). **Advanced maternal age defined as ≥37 years at time of delivery. Other testing indications combined constituted <15% of all indications annually throughout the study period and are not depicted in this figure

TABLE 1 Indication for prenatal diagnosis in cases of confirmed sex chromosome aneuploidies from 2005 to 2020

Indication for prenatal diagnosis, *n* (%)	45,X	47,XXY	47,XXX	47,XYY	Other SCAs[a]	Total
Ultrasound abnormality	174 (59.2)	18 (10.3)	13 (14.3)	6 (11.3)	0 (0.0)	211 (34.2)
Screen positive NIPT	23 (7.8)	100 (57.5)	38 (41.8)	33 (62.3)	3 (60.0)	197 (27.7)
First trimester combined screening	85 (28.9)	19 (10.9)	18 (19.8)	7 (13.2)	1 (20.0)	130 (18.3)
Advanced maternal age	2 (0.7)	26 (14.9)	12 (19.8)	4 (13.2)	1 (20.0)	45 (6.3)
Second trimester serum screening	6 (2.0)	3 (1.7)	4 (4.4)	1 (1.9)	0 (0.0)	14 (2.0)
Other indications[b]	4 (1.4)	8 (4.6)	6 (6.6)	2 (3.8)	0 (0.0)	20 (2.8)
Total	**294**	**174**	**91**	**53**	**5**	**617**

Abbreviations: NIPT: noninvasive prenatal testing; SCA, sex chromosome aneuploidy.

[a]Other SCAs included 48,XXXX; 49,XXXXY; 48,XXXY; 48,XXYY.

[b]Other testing indications included: single gene testing, repeat testing, first trimester serum screening only, high risk screening result (test not specified), no clinical notes given, and 'other' (selective reduction of multifetal pregnancy, maternal anxiety, previous child or fetus with a structural abnormality, previous or recurrent miscarriage).

increase in the prenatal detection of SCA prior to 2016.[4] The subsequent increase likely reflects rapid uptake of NIPT after this time, including screening for SCAs.

Traditionally, only children with SCAs on the more severe end of the spectrum present with clinical features at birth[16] or indeed for medical care at any time across the life course, with up to 75% of males with 47,XXY remaining undetected.[17] Although the clinical phenotype cannot be predicted on the basis of chromosomal findings alone, there is evidence that early detection can be beneficial. Prospective studies of infants with 47,XXY diagnosed by newborn screening identify speech-language delays in 75% and motor skill delays in 50%; early identification and intervention for these infants results in improved neurodevelopmental outcomes.[18,19] It is currently recommended that children with a diagnosis of 47,XXY undergo comprehensive developmental assessments at 9–15 months, 18–24 months and 30–36 months,[20] which can only be facilitated by antenatal or perinatal diagnosis. Antenatal detection also affords the opportunity to actively screen for, and address, somatic outcomes such as the evolution of hypogonadism (pre- and post-puberty) and osteoporosis.[21,22] While interventions to preserve fertility in prepubertal boys with 47,XXY remain controversial,[23,24] early diagnosis does present the opportunity for discussion around potential sperm banking and early intervention in adolescence and early adulthood.[25,26] Prenatal screening for 47,XXY therefore opens up

new possibilities for anticipatory care as well as genetic counselling challenges for individuals, families and health professionals.[27]

Obstetricians have historically not provided pre-test counselling for SCA as the focus of prenatal screening has been the common autosomal aneuploidies (trisomy 21, 13 and 18). Despite the normal or mild phenotype associated with most SCAs, many couples elect to terminate a pregnancy following a prenatal diagnosis of SCA. A systematic review of decisions either to terminate or to continue a SCA-affected pregnancy reported five factors influencing the couple's decision making: the specific type of sex chromosome abnormality, gestational week at diagnosis, parents' age, providers' genetic expertise, and number of children/desire for (more) children.[28] The finding that a providers' genetic expertise is an influence on decision making highlights the need for continuing medical education on these conditions. A 2012 European Society of Human Genetics/American Society of Human Genetics position statement on responsible innovation in prenatal screening highlighted some of these ethical concerns related to patient information and counselling challenges for suspected SCAs.[29]

A limitation of this study is that pregnancy outcomes were not available, meaning that the livebirth prevalence is unknown and the calculations of SCA per 10,000 births cannot adjust for the possibility that many of the SCAs are not liveborn. Our dataset does not include NIPT results per se; but only cases where a positive NIPT result was an indication for prenatal diagnosis. It was not possible to calculate the uptake of NIPT by year as multiple private providers are involved and these data are not collected centrally. We are also unable to assess the overall uptake of prenatal diagnostic testing after a positive NIPT result for a SCA. However, our prior individual patient record linkage study including women undergoing NIPT in 2015 in Victoria found that 58.7% of women with positive NIPT for trisomy 21 had prenatal diagnostic confirmation.[3] Significantly fewer women with positive NIPT for suspected SCA underwent prenatal diagnostic confirmation (36.4%, $p < 0.001$).

The past decade has seen an unprecedented rise in the prenatal diagnoses of SCA on amniocentesis and CVS in our population, driven by NIPT-indicated testing. There is now one prenatal diagnosis of 47,XXY for every 2300 births, though the benefits of screening for SCA remain controversial. It is time for obstetricians and other maternity care providers to be equipped to discuss a diagnosis of 47,XXY in particular, and to have access to appropriate genetics services for post-test counselling and decision support for couples. More prospective research is required to better understand the natural history of SCA in children with a prenatal diagnosis.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

ETHICS STATEMENT

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DATA AVAILABILITY STATEMENT

Permission to access to the de-identified data underlying this study may be requested from the Steering Committee of the VPDD via the Human Research Ethics Committee of the Royal Children's Hospital at rch.ethics@rch.org.au.

ORCID

Cecilia Pynaker https://orcid.org/0000-0003-2360-0781
Lisa Hui https://orcid.org/0000-0002-9720-3562

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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